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PATHOBIOLOGY OF HTLV-III/LAV IN HUMAN MONOCYTE-MACROPHAGE

FINAL REPORT

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) We have studied the pathobiology of human immunodeficiency virus (HIV) in human monocyte-macrophages. The role of cytokines released following HIV infection of these target cells, potentially with exposure to physiologic stimuli such as endotoxin, as well as the effects of cytokines on regulation of HIV replication have been pursued. The biochemical basis of control of HIV replication within monocytes has been examined by studying cell signaling events. In addition, the effects of HIV on hematopoietic stem cells has been explored. These studies may improve our understanding of dysregulation of myeloid cell function in AIDS and thus provide the framework for improved therapeutic strategies for this disease. (JES)					
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FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

The investigator(s) have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.



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Introduction

We have studied the effects of human immunodeficiency virus (HIV) on human monocyte-macrophages. We both focused on the modulation of cytokine production by HIV infected macrophages in response to potentially physiologic stimuli such as endotoxin and studied the effects of cytokines which might be clinically useful in the therapy of AIDS with regard to modulation of HIV infection within these monocytic cells. These studies of mature monocyte-macrophages were done in tandem with studies on bone marrow stem cell development following HIV infection. We thus were able to begin to address the problem of impaired hematopoiesis in patients with AIDS and ARC using in vitro models of bone marrow stem cell development.

Background

The basis of this project rests on a major component of the biology of HIV. It is clear that the monocyte-macrophage is an important in vivo target of HIV, and that efforts to develop both therapeutics and vaccines must take into account the interaction of the retrovirus with this particular myeloid target cell. Furthermore, bone marrow dysfunction is frequent in patients with AIDS and ARC and often the tissue limiting toxicity in the use of anti-retroviral agents such as Zidovudine. An improved understanding of the biology of HIV within monocyte-macrophages should provide important insights into immunology and virology in general and into clinical strategies for AIDS in particular.

Rationale

The rationale used in the current study was to first establish in vitro models with both permanent human cell lines, such as the monocytic cell lines U937 and THP1, and then to study primary monocyte-macrophages derived from peripheral blood or lung. In addition, cultures of human bone marrow from HIV uninfected as well as HIV infected individuals were established and stem cells grown within semisolid matrices. A variety of HIV isolates was employed for these studies, including isolates with tropism for monocytic cells (such as BAL or SF162) as well as isolates with tropism for T cells (such as HIV IIIb and RJ4029).

Hematopoiesis and Cytokines

Our initial studies demonstrated that bone marrow grown from HIV infected individuals who were not on myelosuppressive therapy had normal characteristics in vitro. This strongly suggested that bone marrow stem cells were normally responsive to cytokine modulation in vitro and that the virus was not grossly cytopathic to the progenitor cell. We also explored the effects of candidate antiviral drugs on bone marrow stem cells, and determined that Zidovudine was particularly toxic to erythroid and myeloid progenitors while dideoxyadenosine or dideoxyinosine were not (Figure 1 and 2; Table I). These studies established the ground work for further development of non-marrow toxic nucleoside derivatives which have recently entered clinical trial. In order to better understand the pathogenesis of impaired hematopoiesis, with particular reference to HIV infection of mature

monocyte-macrophages, we studied the production of tumor necrosis factor alpha and interleukin-1 beta by THP1 monocytic cells infected with HIV (Figure 3). We found that there was not a marked abnormality in production of these two cytokines during chronic infection of this permanent monocytic cell line, but there appeared to be differences in response during acute infection to the endogenous stimulus endotoxin. When these initial observations were extended to mature peripheral blood monocyte-macrophages, we found that there were no abnormalities during acute or chronic infection with regard to production of tumor necrosis factor alpha, interleukin-1 beta, interleukin-6, or granulocyte macrophage colony stimulating factor (Figure 4-6; Table II). These studies were done under strictly endotoxin-free conditions and strongly suggested that retroviral infection per se does not lead to abnormal gene expression of these four cytokines. Studies were done with both monocyte tropic HIV isolates and T cell tropic isolates, as well as exposure of monocytic cells to the HIV envelope glycoprotein gp120.

Virus-receptor interactions

Our interest in the interaction of HIV with its cell surface receptor, the CD4 structure, led us to utilize recombinant soluble CD4 in our experiments. This proved to be a particularly useful reagent. We were able to determine that there were different neutralization profiles of different HIV isolates in vitro with recombinant soluble CD4 (Figure 7-11; Table III). Most difficult to neutralize was the HIV-2 ROD isolate, strongly suggesting that this retrovirus related to HIV-1 has a different envelope glycoprotein structure. Recent data suggest that wild type HIV-1 isolates from patients

in the United States have a wide range of neutralization profiles by recombinant soluble CD4. This indicates that the interaction of HIV with its CD4 receptor is more complex than originally thought. Our studies of HIV infection of monocytic cells asked the question whether in such cells, which express the Fc receptor, antibody coating of virus might allow entry independent of binding to soluble CD4 (Figure 12-14). Although we did detect a low degree of enhancement (2-4 fold) of productive HIV infection of U937 monocytic cells in the presence of non-neutralizing concentrations of anti-HIV antibody, we found that recombinant soluble CD4 or the anti-CD4 monoclonal antibody Leu3a completely inhibited infection. This suggested that interaction of HIV with CD4 on the cell surface was necessary for productive infection of monocytic cells.

Molecular studies

In order to further explore the nature of HIV infection on a molecular level, we initiated studies of the temporal aspects of DNA and RNA synthesis during HIV infection (Figure 15). This was first done in T cells and then in monocytic cells. Of note, using the prototype HXB2 clone which was engineered to express an authentic nef gene, we were unable to detect a negative influence on viral growth in T cells or monocytic cells (Figure 16). The function of the nef gene is still not clearly understood.

Antiviral Strategies in Monocyte/Macrophages:

Given the recognized importance of the monocyte/macrophage as a primary target and probable reservoir of HIV infection, we have continued our efforts to define antiviral strategies that are effective in cells of this lineage. To this end, we have examined cell lines with monocytic characteristics such as the U937 cell line but have also developed systems in which to examine normal macrophages in culture. This latter effort has involved the use of human alveolar macrophages as a representative tissue macrophage. This has permitted us to establish a system in which strategies to both prevent de novo infection of macrophages as well as to block transfer of HIV to target lymphocytes could be examined. These systems have been utilized as follows:

- A. Effect of zidovudine and granulocyte-macrophage colony stimulating factor. Alveolar macrophages (AM's) were obtained by bronchial lavage from HIV seronegative normal human volunteers by standard techniques after obtaining written informed consent. The cells were washed and placed in culture at 2×10^6 cells/well in 35 mm six-well plates. Following a 2 hour adherence, the nonadherent cells were washed free and the remaining adherent cells were determined to be >95% macrophages by phagocytosis of 1.1 micron latex beads. The AM monolayers were incubated in medium alone or medium containing 30 units/ml GM-CSF for 48 to 72 hours. After this incubation, the monolayers were washed, incubated with 25 ug/ml DEAE dextran for 30 minutes and infected with the IIIB strain of HIV-1 (RT of inocula = $5.6 \log_{10}$ cpm/ml). After the virus adsorption, cultures that had been pretreated with GM-CSF were reexposed to this compound alone or in combination

with 1 μ M AZT. Cultures that had been incubated in medium alone preinfection were left untreated or were exposed to 1 μ M AZT immediately post infection. Controls included mock infected cultures maintained in medium alone or medium containing 30 units/ml GM-CSF and/or 1 μ M AZT. Drugs were replaced in the culture supernatants with each media change. Ten days postinfection phytohemagglutinin (PHA) stimulated peripheral blood mononuclear leukocytes (PBMLs) from HIV seronegative donors were added to some of the AM cultures as target cells (5×10^5 PBMLs/well). Cultures were then examined for an additional 10 days during which drug was maintained in the treated cultures. To determine the effect of drug removal in this system, cultures that had been maintained for 20 days in the presence of AZT and/or GM-CSF but to which PBMLs had not been previously added were washed free of drug and PHA stimulated PBMLs were added at that point. These cultures were then monitored for an additional 15 days.

In our experience, when normal AM's are infected *in vitro* with the IIIB strain of HIV-1, they exhibit no cytopathic effects and supernatant RT activity falls to low or undetectable levels by 5-7 days postinfection. Supernatant HIV antigen also predictably falls post-HIV exposure but it remains detectable for up to 6 weeks if the cells are left in culture, usually in the range of 100-900 pg/ml. When stimulated target cells such as PHA stimulated PBMLs are added at any point postinfection, a marked rise in RT activity and supernatant HIV antigen is seen within 4 days of their addition. This system, therefore, has been designed

to mimic the "reservoir" function of macrophages for the ongoing infection of target lymphocytes.

In the studies described here, addition of 1 μ M AZT immediately postinfection resulted in significant antiviral activity by days 6 and 10 postinfection. Mean HIV antigen levels in infected control cultures were reduced from 813 (\pm 128) to 180 (\pm 17) pg/ml ($p=.001$), and from 808 (\pm 119) to 22 (\pm 9) pg/ml ($p<.001$) on days 6 and 10, respectively, by AZT alone. RT activities and HIV antigens subsequently fell to undetectable levels in treated cultures and remained undetectable in cultures that received PBMLs as long as AZT was maintained in the medium. In contrast, pretreatment of AM's with GM-CSF at 30 units/ml and maintenance of the cytokine in the cultures resulted in no inhibitory effect on HIV replication but importantly no potentiation of HIV replication occurred. The combination of AZT and GM-CSF demonstrated significant anti-HIV activity in this system to a degree similar to that seen with AZT alone. The combination was also effective in inhibiting transfer of infection to target PBMLs and this effect was equivalent to that seen with AZT alone. Given the marked effectiveness of AZT any additive effectiveness of GM-CSF in this system could not be elucidated. However, no antagonism of the effectiveness of AZT was demonstrated.

We next examined the pattern of HIV expression after drug removal and attempted to determine if virus expression could be observed in the cultures that had been completely suppressed by AZT or AZT plus GM-CSF. To do this, infected AM monolayers that

had been maintained in culture with/without drug exposure for 20 days postinfection were washed thoroughly and PHA stimulated PBMLs were added to the wells. The cultures were monitored for supernatant HIV antigen for 15 days. Supernatant antigen expression briskly rose in infected control and GM-CSF exposed cultures. In previously AZT exposed cultures, virus expression was delayed but still was readily apparent by day 8 postdrug removal. In contrast, in 4 of 6 experiments the combination of AZT plus GM-CSF appear to have a greater antiretroviral effect than AZT alone in that virus expression was not seen after PBML addition and drug removal.

These results demonstrate that AZT at 1 μ M was effective at inhibiting the low level productive infection of AM's and in preventing transfer to stimulated target cells as long as the drug was maintained in the culture system. This confirms that AZT is effective in monocytic cell systems. GM-CSF as a single agent demonstrated no HIV inhibitory activity in AM's, in contrast to what we had previously demonstrated in the U937 cell system. However, most importantly, no potentiation of HIV replication was seen. Further, the relatively lymphocytotropic IIIB strain was not converted to a more monocytotropic strain by exposure to this cytokine. The combination of AZT plus GM-CSF demonstrated anti-HIV activity comparable to AZT alone at the concentrations examined. The degree of effectiveness of AZT precluded the ability to demonstrate an additive or synergistic effect of GM-CSF but perhaps just as importantly no antagonism was demonstrated. The inability to induce virus replication in 4 of 6 AZT plus GM-

CSF treated cultures after drug removal and PBML addition is of interest in that it suggests an additive antiviral effect of the combination. This may well be explained by the reported ability of GM-CSF to increase the anabolic phosphorylation of AZT in monocytes resulting in a lowering of the AZT ED₅₀.

- B. Effect of recombinant soluble CD4: The U937 monocytic cell line and human alveolar macrophages were employed to examine the effectiveness of recombinant soluble CD4 on HIV-1 infection of cells of the monocyte macrophage lineage. rCD4 was obtained from Genentech, Inc., and consisted of the 370 amino acids which comprise the extracellular domain of the molecule. For the acute infection of U937 cells, cell-free virus (10^3 - 10^4 TCID₅₀) or a mock inoculum was incubated with 0, 0.1, 1, or 10 ug/ml rCD4 in 96 well plates for 1 hour at 37°C. Then 5×10^5 U937 cells were added and the mixture incubated for another hour at 37°. The cells were transferred without washing to 2 ml of fresh complete RPMI in a 24 well plate. The same concentrations of rCD4 were maintained in the medium of infected cultures for 21 days. For the acute infection of alveolar macrophages, the AM's were prepared as described above and placed in culture in 35 mm 6-well plates. The HIV inoculum (100 TCID₅₀) or the mock inoculum was incubated with 0, 0.1, 1.0 or 10 ug/ml of rCD4 for 1 hour at 37°C then placed on the AM monolayers and incubated for a second hour at 37°. The cultures were maintained with rCD4 in the medium for 15 days and then 5×10^5 PHA stimulated PBMLs were added to the wells to amplify expression of HIV. rCD4 was either removed or maintained in the cultures at that point. For examination of the

transfer of infection from chronically infected AM's to target PBMLs, AM's were placed in culture and infected with HIV and maintained for 15 days. At that point the monolayers were washed and incubated with 0, 1.0, 10.0 or 200 ug/ml of rCD4 for 1 hour before addition of PHA stimulated PBMLs to the wells. rCD4 was then maintained in the medium throughout the subsequent experiment. In these studies, to examine the importance of cell-to-cell contact, PBMLs were either added directly to the AM monolayers or separated by a Transwell (Costar) membrane with a pore size of 0.4 micron.

The results demonstrated that rCD4 had the ability to block acute HIV-1 infection of U937 cells. Infected control cells without rCD4 had detectable RT activity by day 14, an HIV antigen level > 2,000 pg/ml by day 14, and 40% IFA positive cells by day 28. In contrast, rCD4 at 0.1 ug/ml markedly delayed virus expression with the supernatant HIV antigen level not reaching >2,000 pg/ml until day 28 and RT activity and IFA positivity remaining undetectable until day 35. At concentrations of 1 and 10 ug/ml rCD4 completely prevented acute HIV infection in U937 cells by all parameters examined. Further, no expression of virus was detected by any assay up to 8 weeks after rCD4 was removed from the medium and Southern blot analysis for HIV proviral DNA was negative. rCD4 had no deleterious effect on cell viability in the U937 cell system.

Attempts to block the acute infection of human alveolar macrophages with rCD4 were effective at all concentrations ranging from 0.1 to 10 ug/ml. Even following the addition of PHA

stimulated PBMLs to the AM's on day 15 after infection, no supernatant HIV antigen could be detected in treated cultures. Levels of supernatant HIV antigen were consistently ≥ 500 pg/ml in the infected control culture by day 10 after PBML addition but remained undetectable in all rCD4 treated cultures. The results were equivalent whether or not rCD4 was maintained in the cultures after PBML addition.

In the studies designed to examine the ability of rCD4 to block the transfer of HIV-1 infection from already infected macrophages to target stimulated lymphocytes, 1 ug/ml of rCD4 was found to effectively block this transfer if cell-to-cell contact between the AM's and the PBMLs was prevented by the presence of the Transwell membrane. However, if cell-to-cell contact was permitted, HIV transfer to target PBMLs was not inhibited. At 10 ug/ml, rCD4 showed substantial but still incomplete blockage of virus transfer when cell-to-cell contact occurred reducing supernatant HIV antigen levels > 2 logs on day 10 (from 10^6 to $10^{3.8}$ pg/ml). Despite the continued presence of rCD4 in the culture, these levels continued to rise, however, and by day 14 reached $10^{5.7}$ pg/ml in the absence of the Transwell membrane. In contrast, 10 ug/ml of rCD4 did completely block virus transfer when cell-to-cell contact was prevented. At 200 ug/ml rCD4 could block transfer of infection even in the face of free cell-to-cell contact.

These results demonstrate that rCD4 can be quite effective in preventing the de novo infection of cells of the monocyte/macrophage lineage. Direct cell-to-cell transfer of

virus can also be blocked by rCD4 but at considerably higher concentrations, thus posing a potential challenge to this strategy in certain microenvironments.

HIV Pathogenesis:

In order to learn more about the pathogenesis of HIV at the cellular level, we have begun to examine the interaction of cell signalling events with the control of HIV replication. To that end, we have been examining the effect of calcium channel blockers on HIV replication in a number of cell systems. In our initial studies verapamil at concentrations of 25-75 μ M was found to potentiate HIV-1 replication (IIIB strain) in the CEM and H9 lymphoid cell lines but to delay it markedly in the U937 monocytic cell line. We have conducted a series of experiments to try to determine the mechanism of this effect and to see whether it is related to the known calcium blocking effect of this agent. We have first chosen to elucidate the effect in lymphoid cell systems where enhancement of HIV replication is seen. CD4 and CD4A expression were examined in HIV-infected and -uninfected, verapamil-treated and -untreated CEM cells by incubation with monoclonal OKT4 and OKT4A antibodies, fluorescein labelling with a second antibody, and flow cytometric analysis. To determine the effects of verapamil on the HIV long terminal repeat (LTR), transfection experiments were performed in which plasmid constructs containing the HIV-1 LTR link to the chloramphenicol acetyl transferase (CAT) reporter gene and a mutant LTR-CAT construct containing site directed mutations in both NF κ B binding sites were transfected into CEM cells by standard DEAE dextran methods. The transfections were carried out with cotransfection of a *cat* expression vector. After transfection, the cells were split into two

aliquots with/without 50 μ M of verapamil. After 48 hours the cells were harvested, lysed and CAT activity was determined by standard enzymatic methods and thin layer chromatography. To further elucidate the possible role of NF- κ B electrophoretic mobility shift analyses were performed on CEM cells treated with 50-100 μ M verapamil, 50 ng/ml PMA plus 2 μ g/ml PHA or control medium for 24 hours. Nuclear extracts were prepared, the protein concentration determined, and 5-20 μ g of nuclear protein were incubated with 5,000-10,000 cpm of a radiolabelled NF- κ B probe. Competition experiments were undertaken with 2 unlabelled oligonucleotides, 1 containing the normal NF- κ B binding sequence and the other a 3 base pair mutation in each κ B sequence. The DNA binding reactions were analyzed on a 4% polyacrylamide gel at room temperature. Intracellular calcium measurements were performed in cultures using the Fura-2 intracellular dye with fluorescence measured in a Perkin Elmer LS5B spectrofluorimeter.

The results of these studies can be summarized as follows: There was no effect of verapamil on surface CD4 expression in CEM cells. Thus, it did not appear that the enhancement effect of verapamil was related to an increased CD4 or CD4A expression. Verapamil at 50 μ M was found, however, to have a marked effect on activating the HIV LTR in LTR-CAT transfection assays. The percentage of acetylated chloramphenicol products rose from 0.4% to 5.3% at 0 and 100 μ M verapamil respectively (a 13 fold increase). Similar results were seen with Jurkat cells indicating that the effect was not restricted to CEM cells. The presence of intact NF- κ B binding sites was necessary to observe this enhancing effect of verapamil because the drug had no effect on the expression of an LTR CAT plasmid containing mutations in

both NF-kB binding sites. Cotransfection of the *cat* vector was necessary to observe the drug effect. The importance of NF-kB to verapamil's effect was confirmed by the electrophoretic mobility shift assay which demonstrated marked induction of NF-kB binding activity with 100 uM of verapamil. This effect was equivalent to that seen with combined PHA and PMA treatment. Further specificity of the induced NF-kB binding activity was confirmed in competition experiments.

To determine whether verapamil's induction of NF-kB activity and activation of the LTR could be correlated with its known mechanism of action as a calcium channel blocker, the intracellular calcium levels of CEM cells during acute HIV infection and the influence of verapamil treatment were studied. CEM cells infected with HIV-1 were generally found to have resting calcium levels 20-40% higher than uninfected cells with effects seen within 3 hours after inoculation of the virus. This effect persisted unchanged throughout the period of development of active virus replication. In contrast, 50 uM of verapamil depressed the resting calcium level in both uninfected and infected cells to levels only 30-50% of those in control, uninfected cells. These lower calcium levels were established immediately on exposure to verapamil and persisted unchanged throughout the period of monitoring despite development of detectable virus expression in the infected cells on day 3. Thus, although changes in calcium could be seen both with verapamil treatment and HIV infection the level of virus replication did not directly correlate with the intracellular calcium levels.

These studies have demonstrated that verapamil in high concentrations can potentiate HIV replication in lymphoid cell lines and that this effect is mediated by the induction of NF-kB. The drug's

effect on HIV replication, however, may be unrelated to its known role as a calcium channel blocker and thus another pharmacologic effect in this system may be evident. We are extending these studies to try to determine why the opposite effect of verapamil is seen in the U937 monocytic cell line as well as to determine verapamil's effect in normal human monocyte/macrophages and CD4+ lymphocytes. Investigations such as these can be used to elucidate the relationship of cell signalling events with the control of HIV replication to better understand the pathogenesis of HIV at the cellular level.

Summary

We have made considerable progress addressing certain specific aspects of biology of HIV in monocyte-macrophages. These include the interaction of virus with the cell surface receptor, the early molecular events which occur after viral penetration, the effects of infection on cytokine expression, the importance of infection on monocyte progenitor development, and the regulation of calcium metabolism in these cells. These data should provide a base upon which subsequent study can be performed in this area of AIDS.

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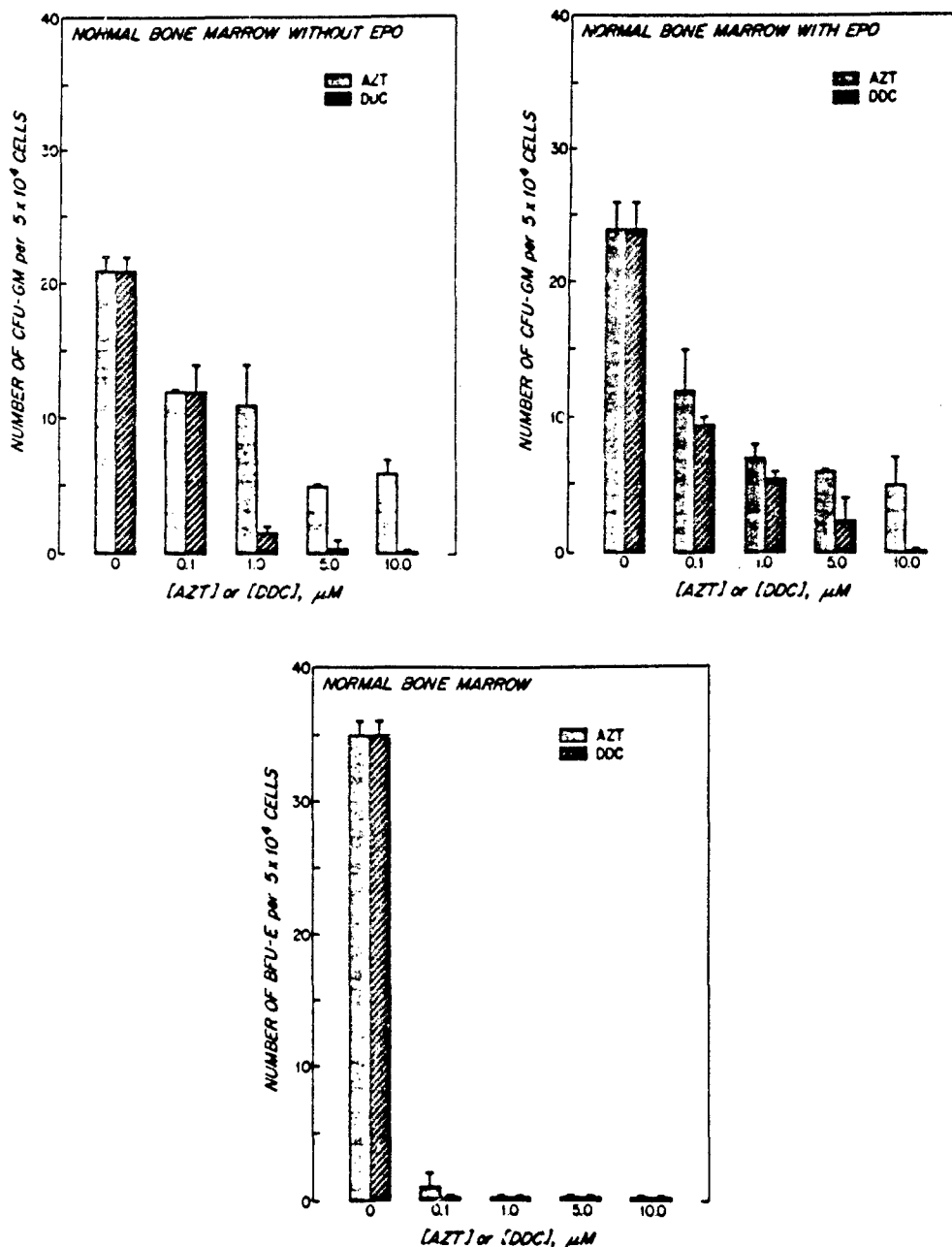


Fig 1. Effects of AZT or DDC on normal bone marrow myeloid and erythroid progenitors. CFU-GM and BFU-E were cultured in methylcellulose in the presence of 1.8 nM rGM-CSF with or without 2 U rEPO and scored on days 12-14. (a) CFU-GM grown without rEPO; (b) CFU-GM with rEPO; (c) BFU-E. Each bar represents the mean colony count of duplicate plates, plus and minus the standard deviation.

Inhibition of Bone Marrow Myelopoiesis and Erythropoiesis in vitro by Anti-retroviral Nucleoside Derivatives

Magaret Johnson, Teresa Caiazzo, Jean-Michel Molina, Robert Donahue, and Jerome E. Groopman

British Journal of Haematology 70:137-141, 1988

Figure 1

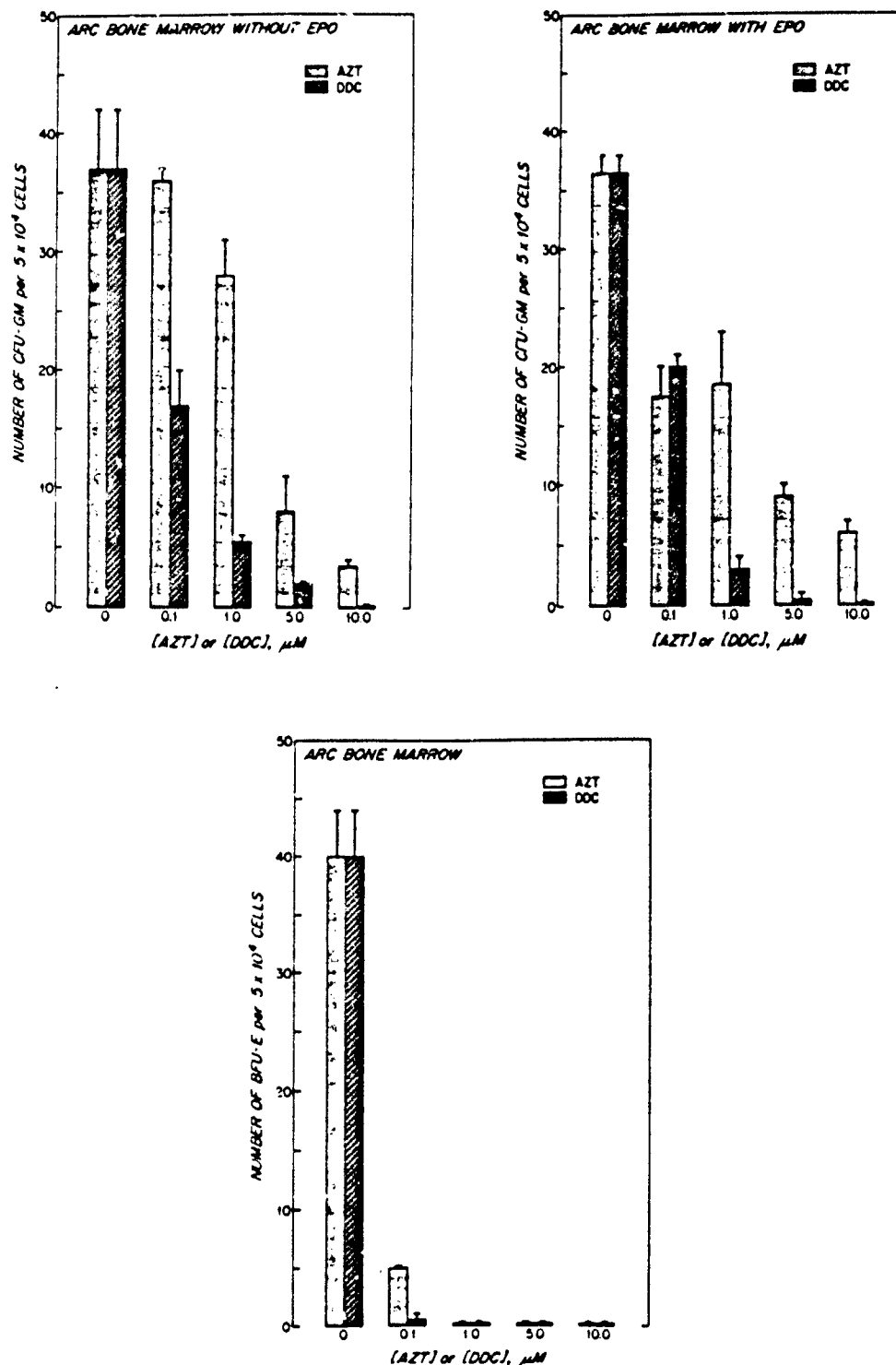


Fig 2. Effects of AZT or DDC on myeloid and erythroid progenitor development from an HIV infected donor with AIDS-related complex (ARC). Methods and data expressed as in Fig 1. (a) CFU-GM grown without rEPO; (b) CFU-GM with rEPO; (c) BFU-E.

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Figure 2

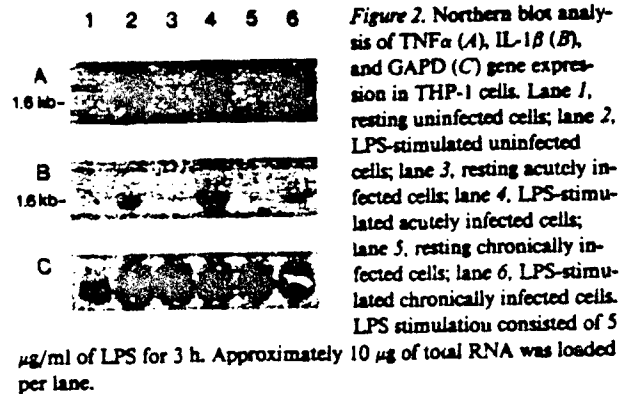


Figure 2. Northern blot analysis of $\text{TNF}\alpha$ (A), $\text{IL-1}\beta$ (B), and GAPD (C) gene expression in THP-1 cells. Lane 1, resting uninfected cells; lane 2, LPS-stimulated uninfected cells; lane 3, resting acutely infected cells; lane 4, LPS-stimulated acutely infected cells; lane 5, resting chronically infected cells; lane 6, LPS-stimulated chronically infected cells. LPS stimulation consisted of 5 $\mu\text{g}/\text{ml}$ of LPS for 3 h. Approximately 10 μg of total RNA was loaded per lane.

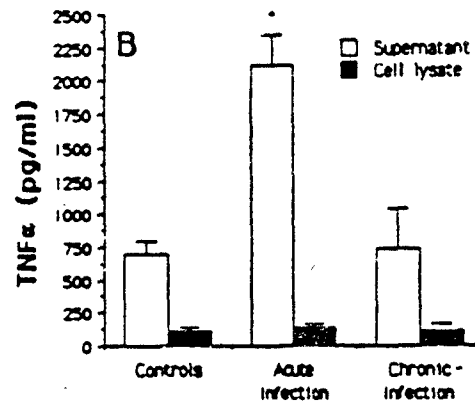
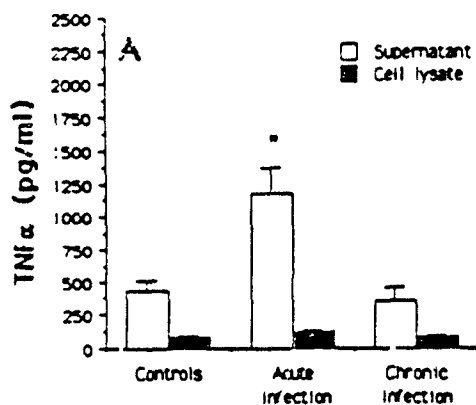


Figure 3. Production of $\text{TNF}\alpha$ by uninfected cells (controls), acutely HIV-infected cells, or chronically HIV-infected cells after stimulation with 5 $\mu\text{g}/\text{ml}$ of LPS (A) or 5 $\mu\text{g}/\text{ml}$ of LPS plus 100 U/ml of $\text{IFN-}\gamma$ (B) for 24 h. Data are presented as mean \pm SEM and are the result of five separate experiments. * $P < 0.001$. † $P < 0.001$.

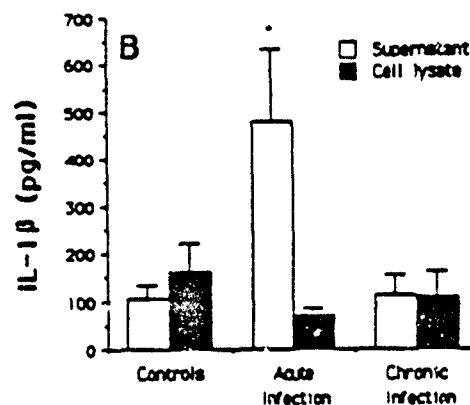
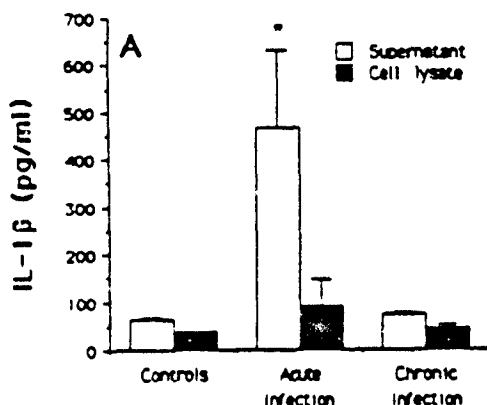


Figure 4. Production of $\text{IL-1}\beta$ by uninfected cells (controls), acutely HIV-infected cells, or chronically HIV-infected cells after stimulation with 5 $\mu\text{g}/\text{ml}$ of LPS (A) or 5 $\mu\text{g}/\text{ml}$ of LPS plus 100 U/ml of $\text{IFN-}\gamma$ (B) for 24 h. Data are presented as mean \pm SEM and are the result of five separate experiments. * $P < 0.01$; * $P < 0.05$.

Production of Tumor Necrosis Factor α and Interleukin 1 β by Monocytic Cells Infected with Human Immunodeficiency Virus

Jean-Michel Molina, David T. Scadden, Randal Byrn, Charles A. Dinarello, and Jerome E. Groopman

J. Clin. Invest. 84:733-737, 1989

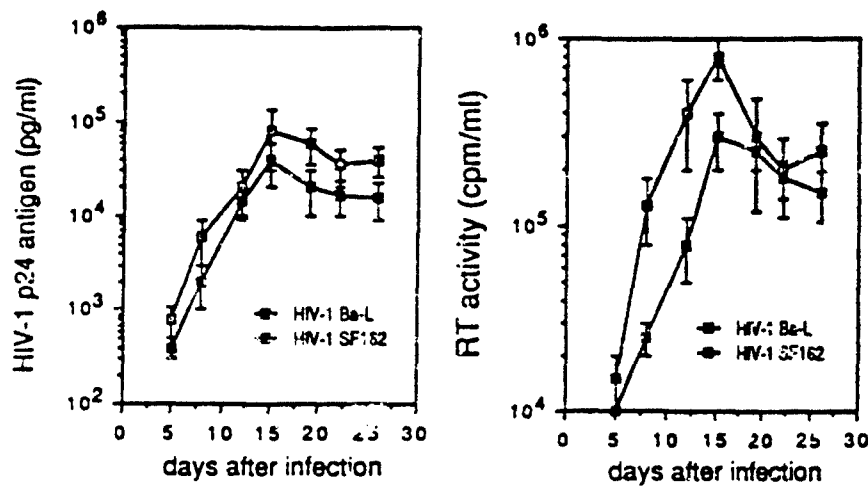


Figure 1. Time course of HIV-1 p24 antigen and reverse transcriptase activity in supernatant of peripheral blood monocytes/macrophages after infection with HIV-1 Ba-L or HIV-1-SF162. Data are mean \pm SE and are result of four experiments.

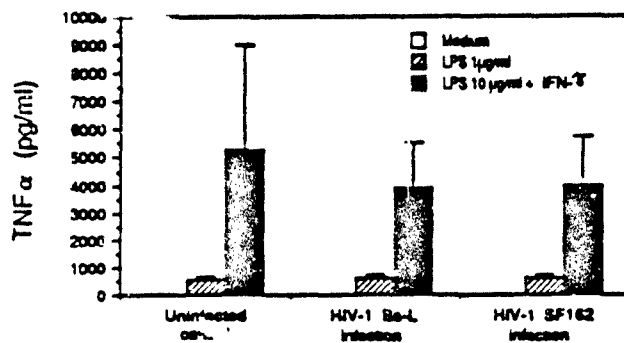


Figure 3. Production of TNF α by peripheral blood monocytes/macrophages after stimulation with LPS (1 μ g/ml) or LPS (10 μ g/ml) plus IFN- γ (100 units/ml) at day 15 of infection with HIV-1 Ba-L or HIV-1 SF162. Data are mean \pm SE and are result of four experiments.

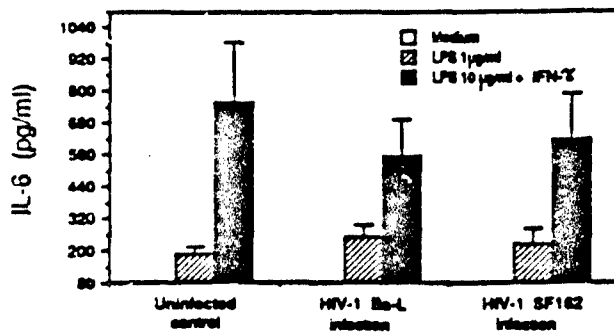


Figure 4. Production of IL-6 by peripheral blood monocytes/macrophages after stimulation with LPS (1 μ g/ml) or LPS (10 μ g/ml) plus IFN- γ (100 units/ml) at day 15 of infection with HIV-1 Ba-L or HIV-1 SF162. Data are mean \pm SE and are result of four experiments.

Production of Cytokines by Peripheral Blood Monocytes/Macrophages Infected with Human Immunodeficiency Virus Type 1 (HIV-1)

Jean-Michel Molina, Ralf Schindler, Roberta Ferriani, Mamoru Sakaguchi, Edouard Vannier, Charles A. Dinarello, and Jerome E. Groopman

Journal of Infectious Diseases: Planned to be published in May 1990

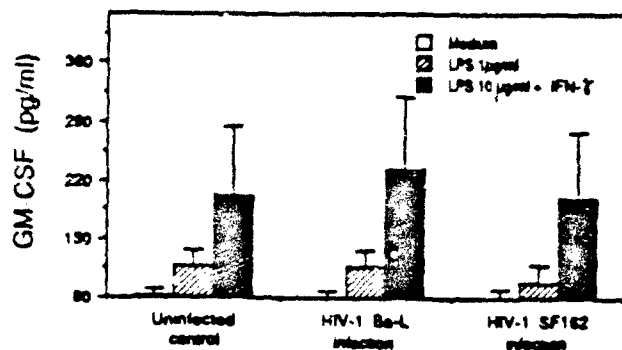


Figure 5. Production of GM-CSF by peripheral blood monocytes/macrophages stimulation with LPS (1 µg/ml) or LPS (10 µg/ml) plus IFN-γ (100 units/ml) at day 15 of infection with HIV-1 Ba-L or HIV-1 SF 162. Data are mean ± SE and are result of four experiments.

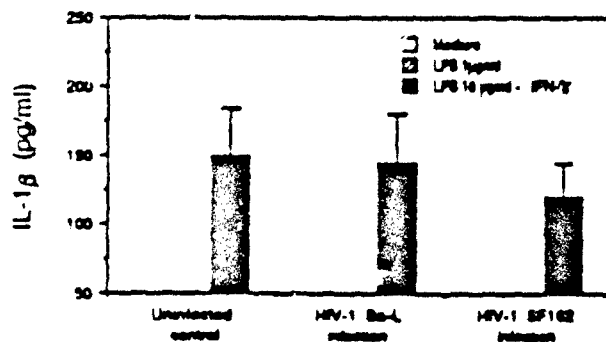


Figure 6. Production of IL-1β by peripheral blood monocytes/macrophages after stimulation with LPS (1 µg/ml) or LPS (10 µg/ml) plus IFN-γ (100 units/ml) at day 15 of infection with HIV-1 Ba-L or HIV-1 SF 162. Data are mean ± SE and are result of four experiments.

Production of Cytokines by Peripheral Blood Monocytes/Macrophages Infected with Human Immunodeficiency Virus Type 1 (HIV-1)

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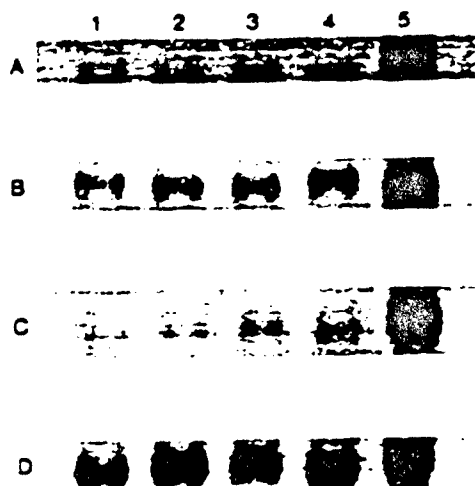


FIG. 2. Northern blot analysis of TNF- α (A), IL-1 β (B), IL-6 (C), and GAPD (D) gene expression in PBMC. A total of 50×10^6 cells were stimulated for 3 h with media alone (lane 1), rgp120 (10 μ g/ml; lane 2), mock shake preparation (10%, vol/vol; lane 3), HIV-1 IIIIB shake preparation (10%, vol/vol; lane 4), or LPS (0.5 μ g/ml; lane 5). Approximately 10 μ g of total RNA was loaded per lane.

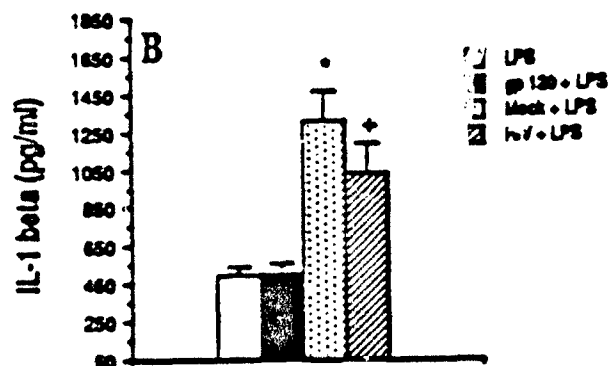
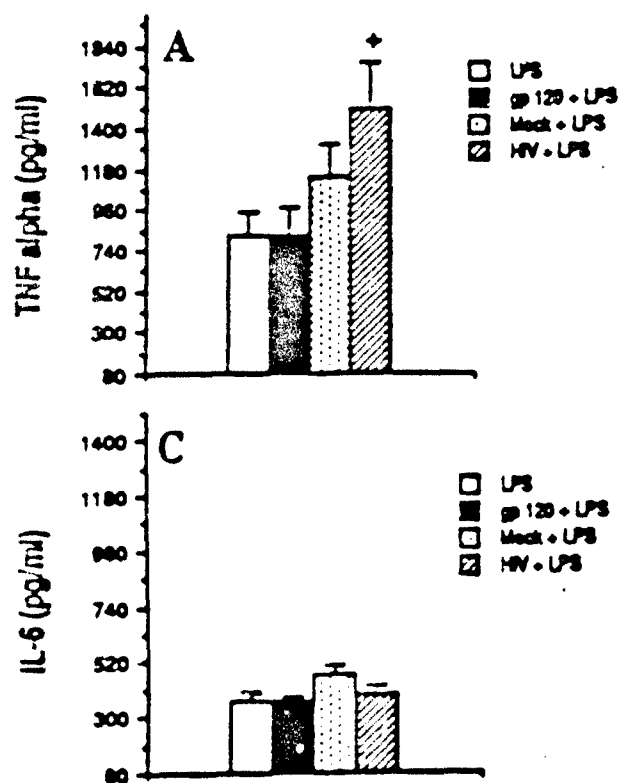


FIG. 3. Production of TNF- α (A), IL-1 β (B), and IL-6 (C) by PBMC. Cells (2.5×10^6 /ml) were exposed to medium alone, rgp120 (1 μ g/ml), H9 mock shake preparation, or HIV-1 shake preparation for 2 h and then stimulated with 0.5 μ g of LPS per ml for 24 h. Total cytokine production (cellular and supernatant) was measured by using RIA. Each datum point is presented as the mean \pm standard error of the mean for five separate experiments (in a single experiment, the range of standard error of the mean was always within 10% of the mean). +, $P < 0.05$; *, $P < 0.01$.

Human Immunodeficiency Virus Does Not Induce Interleukin-1, Interleukin-6, or Tumor Necrosis Factor in Mononuclear Cells

Jean-Michel Molina, David T. Scadden, Charlene Amirault, Annie Woon, Edouard Vannier, Charles A. Dinarello, and Jerome E. Groopman

Journal of Virology: Planned to be published in June 1990

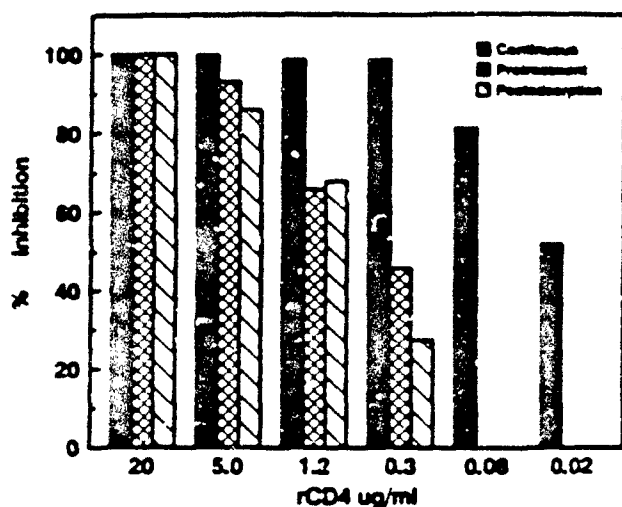


FIG. 1. Recombinant CD4 inhibition of infection by HIV-1 IIIB under continuous, pretreatment, and postadsorption conditions. For continuous-inhibitor conditions, the indicated concentrations of rCD4 were present throughout the 7-day culture period. The extent of infection was determined by measuring supernatant RT activity after 7 days in culture. See Materials and Methods for details of the infection procedure. The bars represent the mean results of three independent experiments. The mean positive control (no rCD4) RT value was 3.8×10^5 cpm/ml. For pretreatment conditions, HIV-1 IIIB was preincubated with rCD4, target H9 cells were added, and after incubation free rCD4 and virus were removed by washing. The cells were then cultured for 7 days. The bars represent the mean inhibition of RT activity in two separate experiments. The mean positive control was 1.5×10^5 cpm/ml. For postadsorption conditions, HIV-1 IIIB was incubated with H9 cells at 4°C and free virus was removed by washing. rCD4 at the indicated concentrations was added to the HIV-cell complex, incubated for 1 h at 4°C, and then removed by washing. The cells were then cultured for 7 days. The bars represent the mean inhibition of RT activity observed after 7 days in two separate experiments. The mean positive control RT value was 1.5×10^5 cpm/ml.

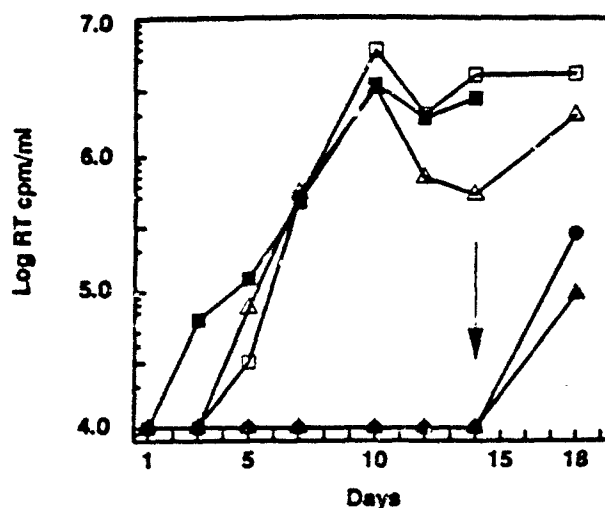


FIG. 3. Inhibition of cell-to-cell spread of HIV-1 by rCD4. Chronically infected H9 cells (HIV-1 strain IIIB) were mixed with uninfected cells at an infected-to-uninfected-cell ratio of 1:10⁵ in the presence of control medium (■), 0.01 µg of rCD4 per ml (Δ), 0.1 µg of rCD4 per ml (●), 1.0 µg of rCD4 per ml (◆), or 10.0 µg of rCD4 per ml (▼). On day 14 (arrow), all cultures were washed to remove rCD4 and suspended in control medium.

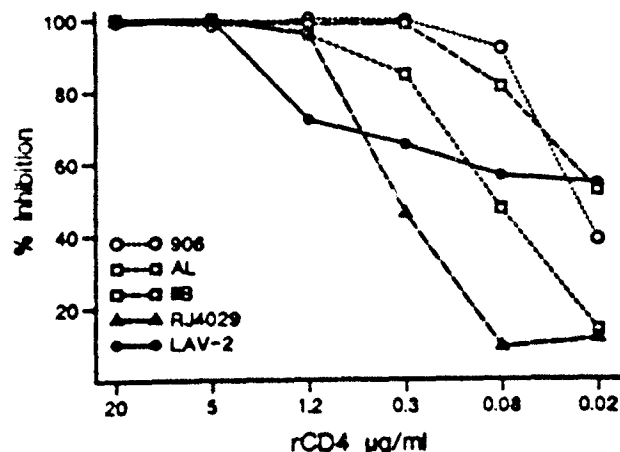


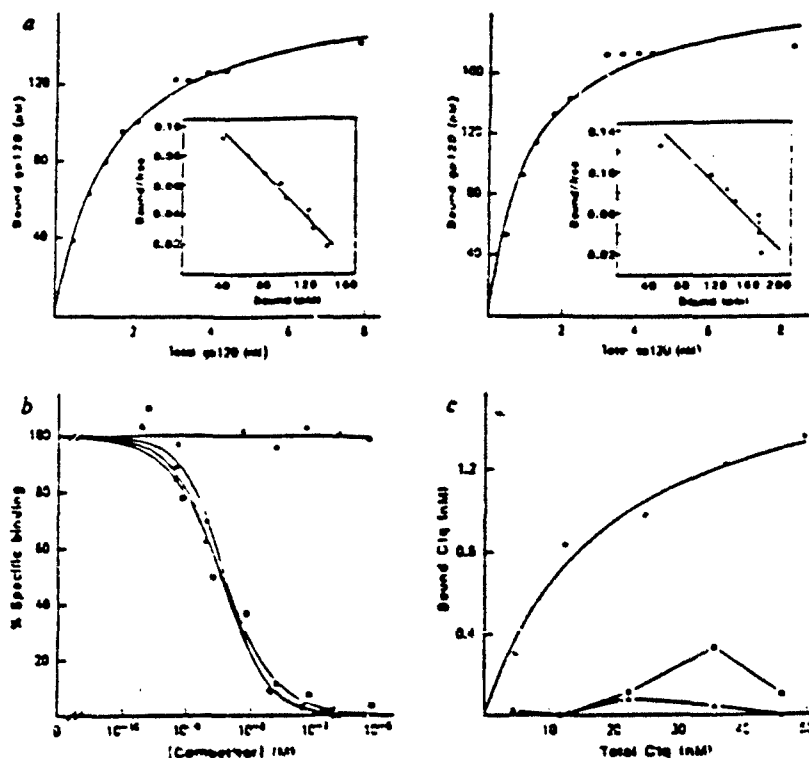
FIG. 4. rCD4 inhibition of infection by HIV-1 strains and HIV-2. H9 cells were infected with 100 TCID₅₀ of each viral strain in the presence of different concentrations of rCD4 (continuous-inhibition conditions; see Materials and Methods). Infection was monitored by measuring supernatant RT activity after 7 days in culture. The results for HIV-1 IIIB are those presented in Fig. 1. HIV-1 isolates are 906, AL, IIIB, and RJ4029. LAV-2 is an HIV-2 isolate. RT activities in the positive controls were as follows: 906, 1.0×10^6 cpm/ml; AL, 1.4×10^6 cpm/ml; 3.8×10^5 cpm/ml; RJ4029, 6.8×10^5 cpm/ml; LAV-2, 2.6×10^5 cpm/ml.

Characterization of In Vitro Inhibition of Human Immunodeficiency Virus by Purified Recombinant CD4

Randal A. Byrn, Iwao Sekigawa, Steven M. Chamow, Jennifer S. Johnson, Timothy J. Gregory, Daniel J. Capon, and Jerome E. Groopman

Journal of Virology, 63:4370-4375, 1989

Fig. 3 Binding properties of CD4 immunoadhesins. **a**, Gp120 saturation binding analysis of CD4 immunoadhesins. Immunoadhesin proteins 4y1 (left) or 2y1 (right) in transfected cell supernatants were incubated with increasing concentrations of purified soluble rgp120 (ref. 50) radiolabelled with lactoperoxidase. The lines drawn for the binding curves and for the Scatchard plots of the data (shown in the insets) represent the best fit as determined by unweighted least-squares linear regression analysis. Dissociation constants calculated from these results and from binding studies of gp120 to soluble rCD4 performed in parallel are given in Table 1. **b**, Binding of CD4 immunoadhesins to Fcγ receptors on U937 cells. Competitive binding analysis was carried out by mixing 0.1 μg ml⁻¹ of ¹²⁵I-labelled human IgG1 (Calbiochem) with increasing concentrations of purified human IgG1 (solid circle), 2y1 (solid square), 4y1 (solid triangle), or soluble rCD4 (open circle) proteins. Curves drawn represent the best fit as determined by unweighted least-squares nonlinear (IgG1, 2y1 and 4y1) or linear (rCD4) regression analysis. Dissociation constants calculated from these results are shown in Table 1. **c**, C1q saturation binding analysis of CD4 immunoadhesins. Purified anti-gp120 IgG2a mouse monoclonal antibody (solid circle), 2y1 (solid square), or 4y1 (solid triangle) proteins were aggregated by binding to gp120-coupled Sepharose, and incubated with increasing concentrations of purified human C1q (Calbiochem) radiolabelled with lactoperoxidase. The curve drawn for the anti-gp120 monoclonal antibody (mAb) represents the best fit as determined by least-squares nonlinear regression analysis; the dissociation constant for C1q binding to this gp120-aggregated anti-gp120 mAb was $\sim 1.8 \times 10^{-6}$ M. **Methods.** **a**, Gp120 saturation binding analysis was carried out as described⁷ except that gp120-CD4 immunoadhesin complexes were collected directly onto Pansorbin; binding was comparable to that observed when complexes were collected with OKT4A as for soluble rCD4. Specifically bound ¹²⁵I-labelled gp120 was determined from the difference in binding in the presence or absence of a 1,000-fold excess of unlabelled rgp120 and is plotted against the total ¹²⁵I-labelled gp120 concentration. **b**, FcR binding analysis was done essentially as described²⁷ except that after centrifugation free IgG1 was removed by aspiration of the aqueous and oil layers. Mixtures of ¹²⁵I-labelled human IgG1 and IgG1, CD4 immunoadhesins or soluble rCD4 were incubated with U937 cells (2×10^6 cells per tube) for 60 min at 4 °C. Specific binding was calculated by subtracting residual nonspecific binding (<25% of specific binding) which could not be competed out by a 1,000-fold excess of unlabelled human IgG1. **c**, C1q binding analysis was done essentially as described²⁸, except that gp120 coupled to CNBr activated Sepharose 6B (Pharmacia) was used as the solid support to aggregate CD4 immunoadhesins or the anti-gp120 mouse mAb. Proteins were adsorbed to gp120 coupled-beads, incubated with varying concentrations of ¹²⁵I-labelled C1q, and bound and free C1q were then separated by centrifugation through 20% sucrose. Specific binding was determined from the difference in binding in the presence or absence of added antibody or immunoadhesin. All data analysis was carried out using the Inplot and Scatplot programs (R. Venable, Genentech). Scatplot was modified from the Ligand program (P. Muncy, NIH).



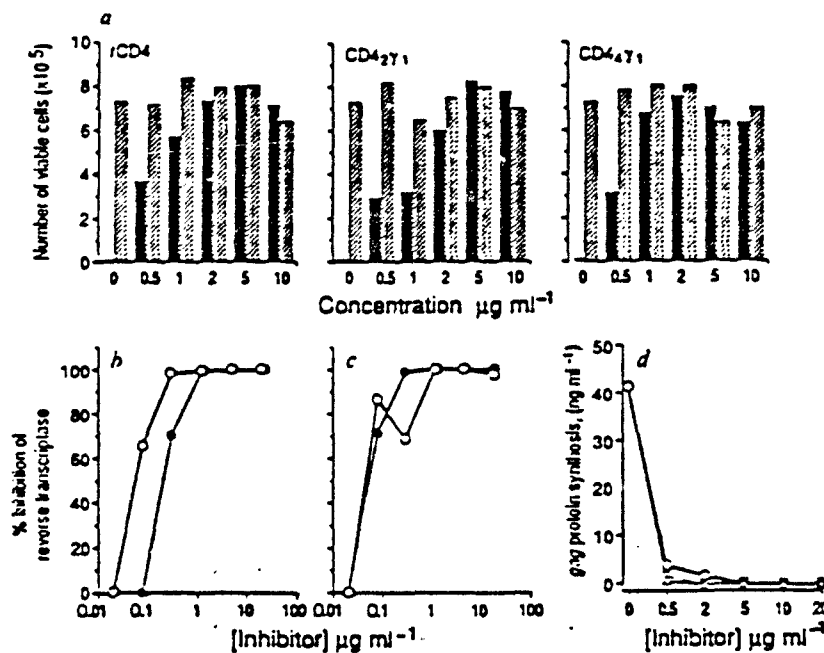
Designing CD4 Immunoadhesins for AIDS Therapy

Daniel J. Capon, Steven M. Chamow, Joyce Mordenti, Scot A. Marsters, Timothy Gregory, Hiroaki Mitsuya, Randal A. Byrn, Catherine Lucas, Florian M. Wurm, Jerome E. Groopman, Samuel Broder, and Douglas H. Smith

Nature, 337:525-531, 1989

Figure 8

Fig. 5 Inhibition of HIV-1 infectivity by CD4 immunoadhesins and soluble rCD4. *a*, Inhibition of the cytopathic effects on ATH8 cells by HIV-1 was examined as described³² with the HTLV-III_B isolate³¹. The number of viable cells at day 10 after infection is shown for varying concentrations of each molecule in the presence (solid bars) or absence (shaded bars) of added virus. The absence of an effect of each CD4 analogue on cell number in the absence of virus indicates that none of these molecules inhibited cell growth. *b*, Inhibition of infection of H9 cells by HIV-1 was carried out as described⁷ with the HTLV-III_B isolate. Reverse transcriptase activity was determined 7 days after infection and is given as the percentage of the level seen in the absence of inhibitor. Solid and open circles represent 2y1 and 4y1, respectively. *c*, Inhibition of infection of U937 cells by HIV-1 (HTLV-III_B isolate) was carried out as described above for H9 cells. *d*, Inhibition of infection of fresh human monocytes by the monocytopathic HIV-1 isolate Ba-L (ref. 35). HIV-1 replication was determined by measuring the level of p24 gag antigen synthesis 10 days after infection using a commercial assay kit (Dupont). Circles, inverted triangles and triangles represent inhibition of p24 synthesis by soluble rCD4, 2y1 and 4y1, respectively.



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Nature, 337:525-531, 1989

Figure 9

CD4



Soluble rCD4



IgG1 Heavy Chain



CD4 Immunoadhesin

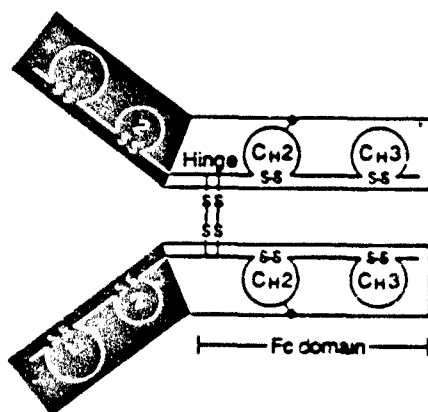


Fig. 1. Structure of CD4 immunoadhesin, soluble rCD4 and the parent human CD4 and IgG1 heavy chain molecules. CD4- and IgG1-derived sequences are indicated by shaded and unshaded regions, respectively. The Ig-like domains of CD4 are numbered 1 to 4; TM and CYT refer to the transmembrane and cytoplasmic domains. Soluble rCD4 is truncated after proline 368 of the mature CD4 polypeptide¹⁴. The variable (VH) and constant (CH1, hinge, CH2, and CH3) regions of IgG1 heavy chain are shown. Disulfide bonds are indicated by (S-S). CD4 immunoadhesin consists of residues 1 to 180 of the mature CD4 protein fused to IgG1 sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region¹⁵) which is the first residue in the IgG1 hinge after the cysteine residue involved in heavy-light chain bonding. The CD4 immunoadhesin shown, which lacks a CH1 domain, was derived from a CH1-containing CD4 immunoadhesin⁵ by oligonucleotide-directed deletional mutagenesis¹⁶, expressed in Chinese Hamster ovary (CHO) cells and purified to >99% purity using protein A-Sepharose chromatography as described⁵.

Biological Properties of a CD4 Immunoadhesin

Randal A. Byrn, Joyce Mordenti, Catherine Lucas, Douglas Smith, Scot A. Marsters, Jennifer S. Johnson, Steven M. Chamow, Florian M. Wurm, Timothy Gregory, and Jerome E. Groopman

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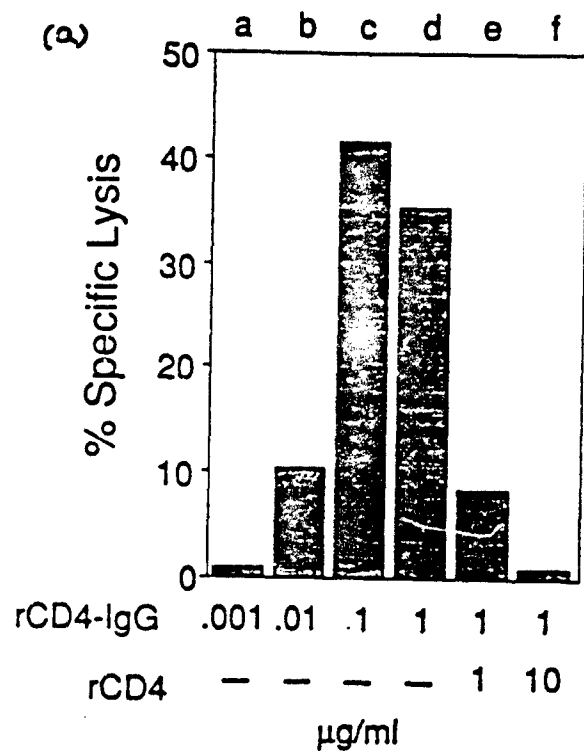
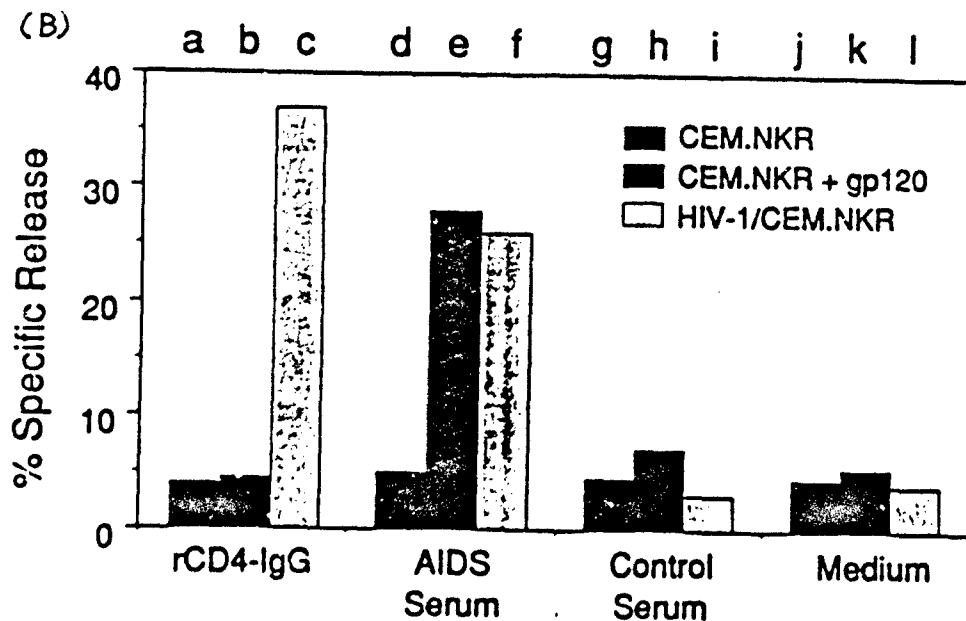


Fig. 2. Antibody-dependent cell-mediated cytotoxicity (ADCC) by CD4 immuno-adhesin. CEM T-lymphoblastoid target cells were labeled with ^{51}Cr , incubated with rCD4-IgG, rCD4, serum, or control media for 30 min, and mixed with peripheral blood mononuclear cells (PBMC) effector cells at an effector to target ratio of 50:1. The cell mixtures were incubated for 20 h at 37°C , and the cell-free supernatant was collected and assayed for ^{51}Cr released from target cells. A) HIV-1 infected CEM.NKR target cells, as by effector cells in the presence of rCD4 immuno-adhesin at 0.001 (lane a), 0.01 (lane b), 0.1 (lane c) and 1.0 $\mu\text{g/ml}$ (lanes d-f). Also shown is the blocking by rCD4 at 1.0 (lane e) and 10 $\mu\text{g/ml}$ (lane f) of target cell lysis mediated by 1.0 $\mu\text{g/ml}$ CD4 immuno-adhesin. The level of cell lysis observed with CD4 immuno-adhesin was comparable to that mediated by a control AIDS patient serum. rCD4 itself does not mediate target cell lysis at concentrations up to 10 $\mu\text{g/ml}$. Uninfected CEM.NKR targets were not lysed by effector cells in the presence of CD4 immuno-adhesin, AIDS patient serum or normal human serum (see below), but could be lysed in the presence of a rabbit anti-rCD4 serum (not shown). B) ADCC of uninfected CEM.NKR target cells (lanes a, d, g and j), uninfected CEM.NKR cells incubated with soluble gp120 (ref. 17) (lanes b, e, h and k) and HIV-1-infected CEM.NKR (lanes c, f, i and l) mediated by CD4 immuno-adhesin (lanes a-c), AIDS patient serum at 1/1000 final dilution (lanes d-f), serum from an uninfected individual at 1/1000 final dilution (g-i) and complete medium (lanes j-l).



Biological Properties of a CD4 Immuno-adhesin

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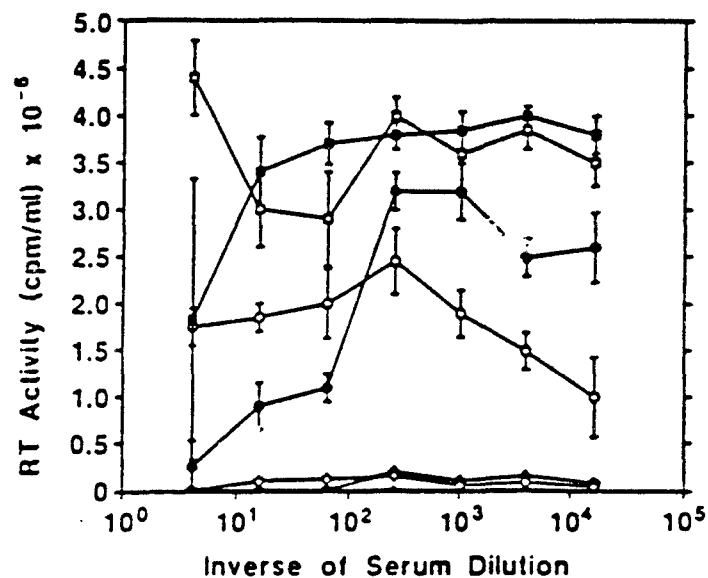


FIG. 1. Kinetics of U937 enhancement of infection by HIV-1 antibody-positive serum. U937 cells were infected by 100 TCID₅₀ HIV-1 in the presence of serial dilutions of serum from an HIV-1 antibody-positive patient (closed symbols) or an HIV-1 antibody-negative healthy control (open symbols). Virus production was detected by supernatant RT activity on Days 7 (▲), 10 (◆), 13 (●), and 16 (■) after infection. Bars reflect standard error of the mean.

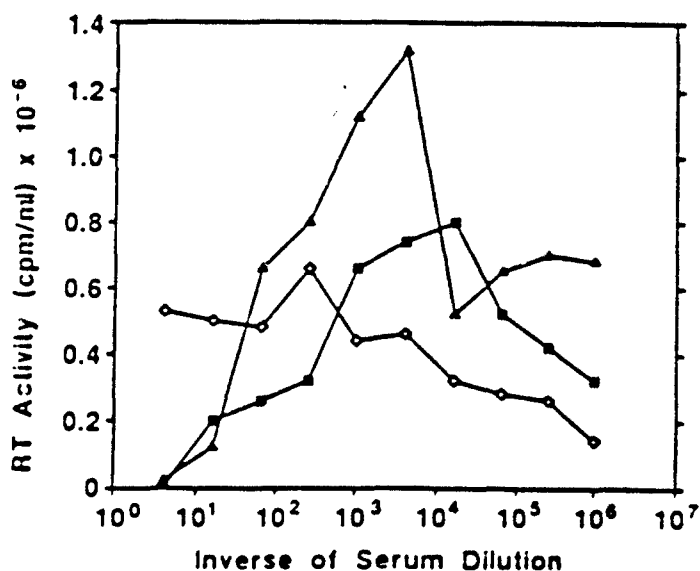


FIG. 2. HIV-1 antibody-positive serum enhancement of HIV-1 infection. U937 cells were infected by 100 TCID₅₀ HIV-1 in the presence of serial dilutions of serum from an HIV-1 antibody-positive asymptomatic (▲), ARC (■) patient, and serum from HIV-1 antibody-negative healthy individual (○) as a control. Virus production was detected by RT supernatant activity on Day 13 after infection.

Inhibition of Serum-Enhanced HIV-1 Infection of U937 Monocytoid Cells by Recombinant Soluble CD4 and Anti-CD4 Monoclonal Antibody

Michael Zeira, Randal A. Byrn, and Jerome E. Groopman

AIDS Research and Human Retroviruses: Planned to be published
6:629-639, 1990

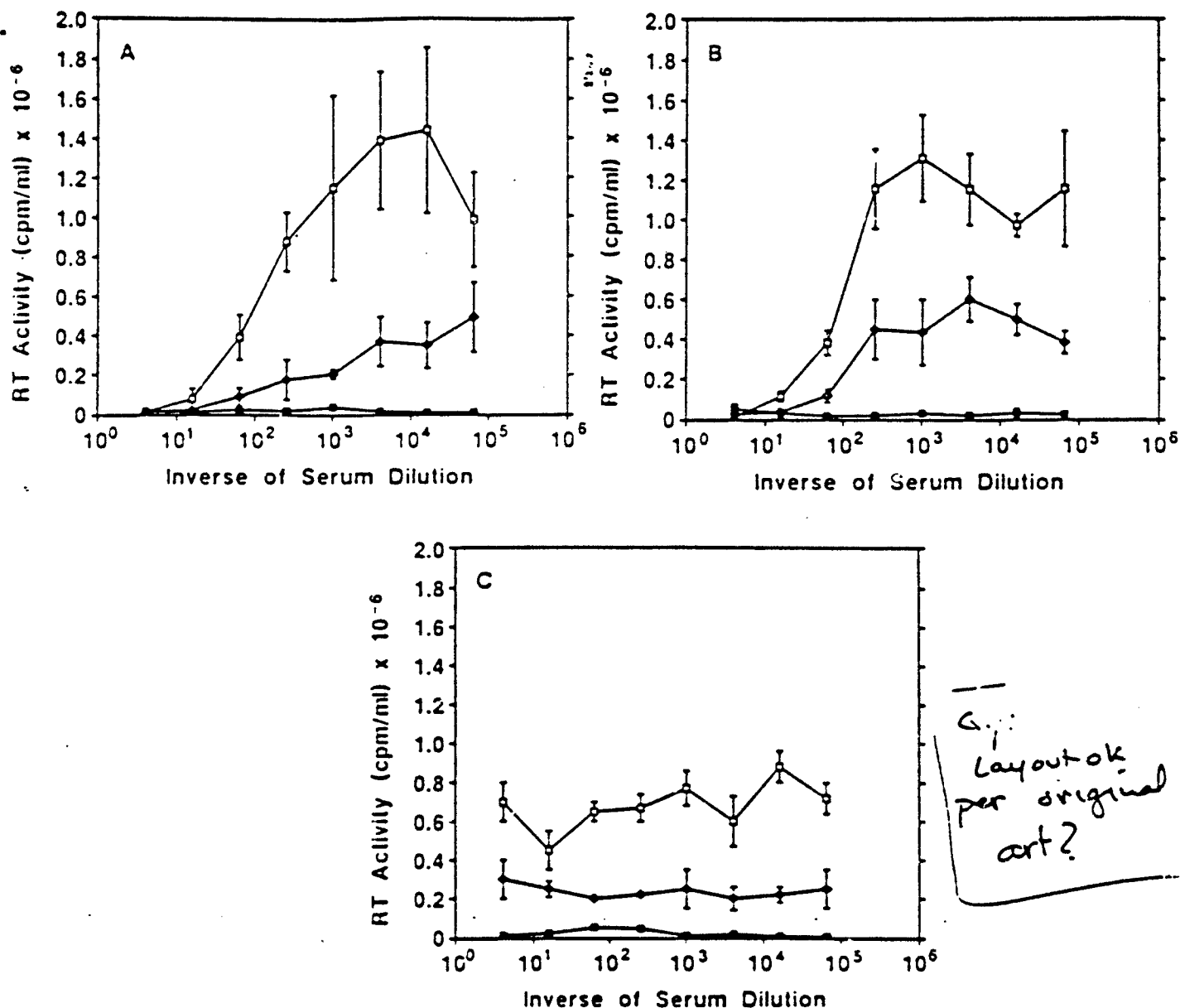


FIG. 3. Inhibition of HIV-1 infection by CD4. 100 TCID₅₀ of HIV-1 were preincubated for 1 h at 4°C with rCD4 at 0.1 $\mu\text{g/ml}$ (\blacklozenge), 1.0 $\mu\text{g/ml}$ (\blacksquare), or control medium (\circ), in the presence of serial dilutions of serum from an asymptomatic ~~pp~~ ^{1. AIDS patient} HIV-infected patient (A) and AIDS patient (B). U937 cells were then added as described in Materials and Methods. Supernatant RT activity was assayed on Day 13 of culture.
 or an HIV-seronegative individual (C).

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Inhibition of Serum-Enhanced HIV-1 Infection of U937 Monocytoid Cells By Recombinant Soluble CD4 and Anti-CD4 Monoclonal Antibody

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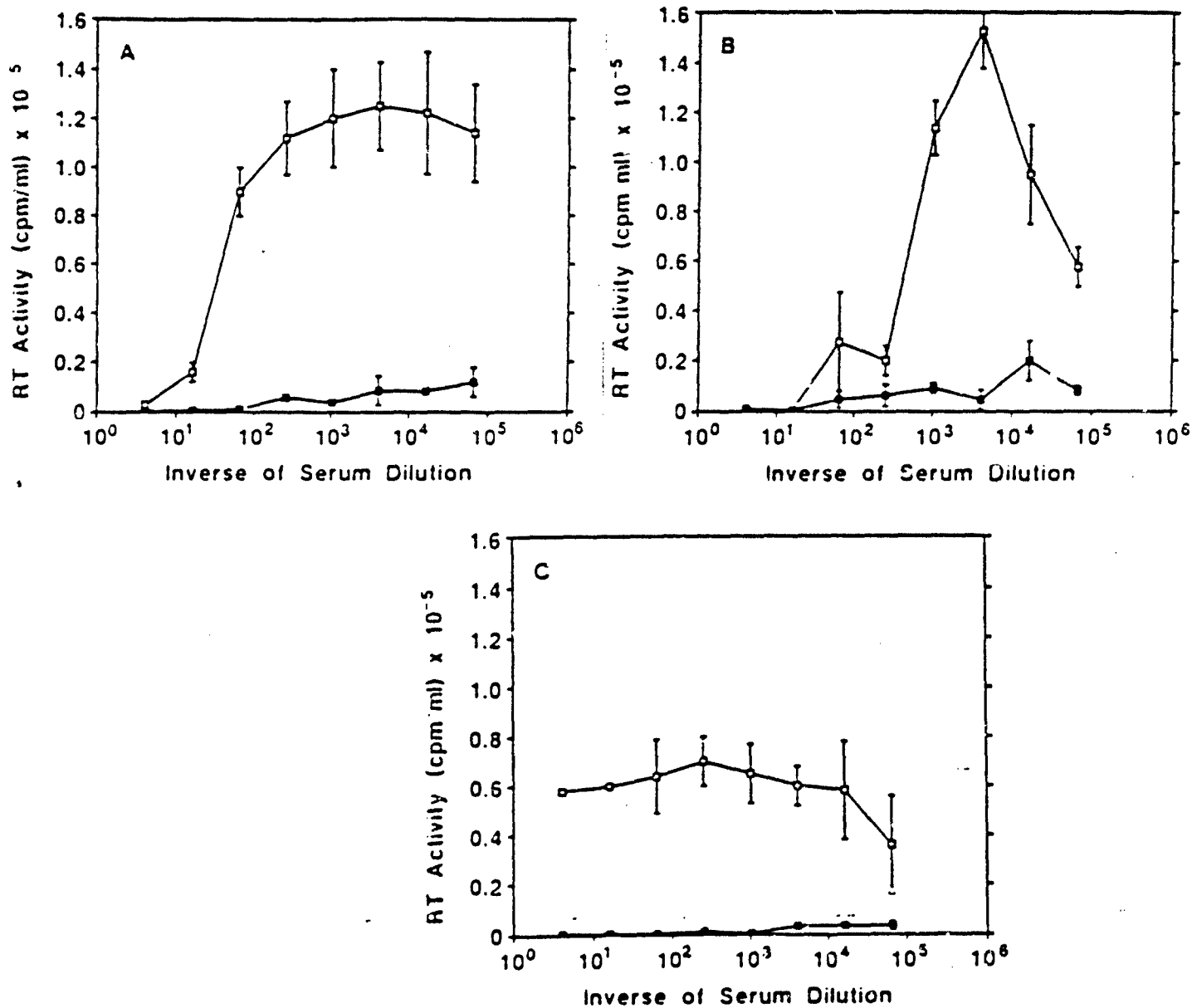


FIG. 4. Inhibition of HIV-1 infection by Leu3a. U937 cells were preincubated with 1 μ g/ml anti-Leu3a (■) or complete media as control (□). 100 TCID₅₀ HIV-1 was incubated with serial dilutions of serum from an asymptomatic HIV-1-infected patient (A), an AIDS patient (B), or a healthy HIV-seronegative individual (C), in parallel to the U937 preincubation. The anti-Leu3a-treated cells were mixed with the anti-HIV-treated virus, incubated for 1 h at 37°C, and transferred to 24-well plates. Supernatant RT activity was assayed on Day 13 after infection.

Inhibition of Serum-Enhanced HIV-1 Infection of U937 Monocytoid Cells by Recombinant Soluble CD4 and Anti-CD4 Monoclonal Antibody

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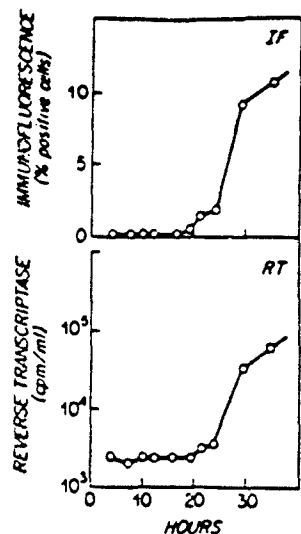


FIG. 1. One-step growth curve of HIV. IF, Change in the percentage of immunofluorescent-positive cells during the course of infection; RT, change in reverse transcriptase activity in cell culture supernatant.

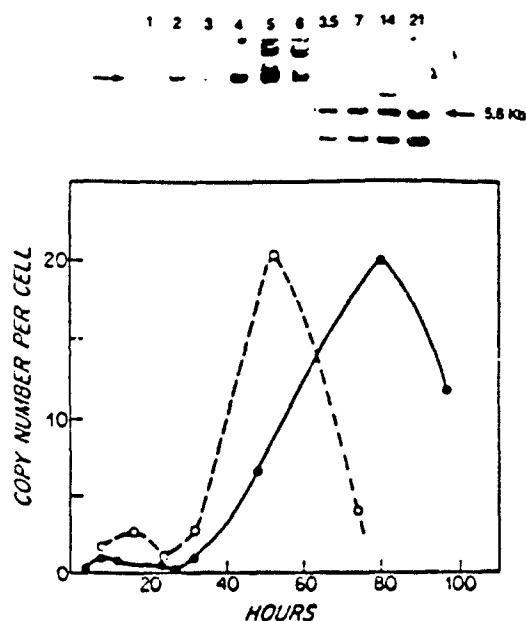


FIG. 3. Amounts of unintegrated HIV DNA in infected cultures. Top. Determination of copy number. One example of Southern blot hybridization is shown. The first six lanes show DNA samples prepared at various times after infection. The last four lanes indicate DNA concentrations (in picograms) of the 5.6-kb *SacI* fragment of the plasmid containing the HIV genome. Bottom. Changes in the copy number per exposed cell during the course of infection. Two representative infections are shown. They are different in the percentage of initially infected cells determined by indirect immunofluorescence, 2% (●) and 8% (○), respectively.

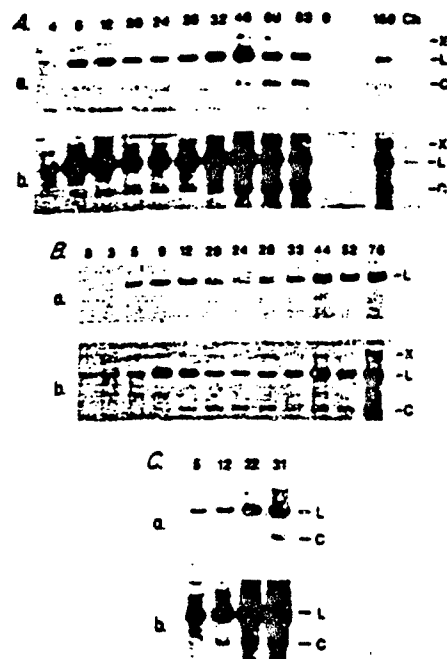


FIG. 2. Hybridization analysis of HIV-specific low-molecular-weight DNA. (A) Total low-molecular-weight DNAs. Short (a) and long (b) exposures of the same autoradiogram are shown. (B) Cytoplasmic (a) and nuclear (b) low-molecular-weight DNAs. (C) Low-molecular-weight DNA from infected primary T cells. Short (a) and long (b) exposures of the same autoradiogram are shown. Numbers at the top of lanes are hours after the infection was started. Lane Ch shows a DNA sample from a chronically infected culture. L, C, and X, Linear, circular, and nicked circular DNAs, respectively. H9 cells or primary T cells (enriched for CD4⁺ cells) were infected with HIV-1 W13, and low-molecular-weight DNA was prepared by Hirt extraction (8) and subjected to Southern blot analysis (24). Cytoplasmic and nuclear fractions were prepared from the same cells by using the detergent Nonidet P-40 and then subjected to Hirt extraction. Because of limits in Hirt extraction, it is difficult to quantitatively compare the amounts of linear DNA in the cytoplasm and the nucleus.

Temporal Aspects of DNA and RNA Synthesis During Human Immunodeficiency Virus Infection: Evidence for Differential Gene Expression

Sunyoung Kim, Randal Byrn, Jerome E. Groopman, and David Baltimore

Journal of Virology, 63:3708-3713, 1989

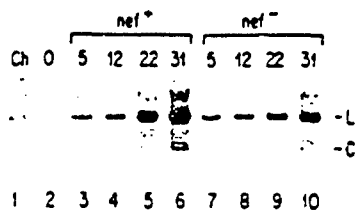


FIG. 2. Comparison of HIV DNA synthesis between the *nef*⁺ and *nef*⁻ strains. Primary T cells enriched for CD4⁺ cells were infected with the *nef*⁺ and *nef*⁻ strains of HIV-1-W13, and low molecular weight DNA was prepared by Hirt extraction (23) and subjected to Southern blot analysis (24). The DNA probe used for hybridization was the Sac I fragment of pW13, which included most of the HIV genomic DNA. Numbers above lanes indicate hours postinfection. L and C are linear and circular DNA, respectively. Lane 1 (Ch) contained control linear DNA of HIV.

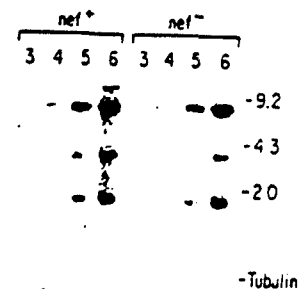


FIG. 3. Comparison of HIV RNA synthesis between the *nef*⁺ and *nef*⁻ strains. Total RNA was prepared from H9 cells infected with the *nef*⁺ and *nef*⁻ strains of HIV-1-W13 and was subjected to RNA blot analysis. (Upper) The DNA probe used for hybridization was the 511-base-pair *Bgl* II fragment of pW13, which includes the polyadenylation signal sequence. Numbers above lanes indicate days postinfection, while those on the right side show the approximate sizes (kb) of HIV mRNA. (Lower) As a control for variation in amount of RNA loaded, the same filter was hybridized with the 1.7-kb *Pst* I fragment of tubulin cDNA (25).

Lack of Negative Influence on Viral Growth by the *nef* Gene of Human Immunodeficiency Virus Type 1

Sunyoung Kim, Kenji Ikeuchi, Randal Gryn, Jerome E. Groopman, and David Baltimore
Proc. Natl. Acad. Sci. USA, 86:9544-9548, 1989

Table I

Mean per cent inhibition (\pm standard deviation)

DDA 2'3'-dideoxyadenosine concentration (μ M)	CFU-GM with EPO	CFU-GM without EPO	BFU-E
0.01	22 \pm 27	17 \pm 16	21 \pm 31
0.05	16 \pm 29	15 \pm 21	12 \pm 22
0.1	21 \pm 18	39 \pm 10	27 \pm 14
1.0	34 \pm 30	9 \pm 15	44 \pm 42
5.0	9 \pm 9	23 \pm 20	32 \pm 38
10.0	20 \pm 20	27 \pm 10	25 \pm 32
20.0	35 \pm 15	46 \pm 7	63 \pm 36
30.0	25 \pm 12	36 \pm 25	38 \pm 28
40.0	32 \pm 22	47 \pm 14	42 \pm 41
50.0	45 \pm 18	47 \pm 14	58 \pm 24
75.0	49 \pm 31	53 \pm 7	61 \pm 30
100.0	70 \pm 21	70 \pm 17	90 \pm 8

Results represent the mean per cent inhibition (\pm standard deviation) for $n=4$ bone marrow donors.

Inhibition of Bone Marrow Myelopoiesis and Erythropoiesis in vitro by Anti-retroviral Nucleoside Derivatives

Margaret Johnson, Teresa Calazzo, Jean-Michel Molina, Robert Donahue, and
Jerome E. Groopman

British Journal of Haematology, 70:137-141, 1988

Table I

Table II

Production of TNF- α , IL-1 β , and IL-6 by PBMC
exposed to various stimuli^a

Stimulus and concn	Mean \pm SEM concn (pg/ml) of cytokine		
	TNF- α	IL-1 β	IL-6
Control	194 \pm 31	87 \pm 12	108 \pm 17
LPS (0.5 ng/ml)	1,766 \pm 828	496 \pm 47	368 \pm 30
rgp120 (μ g/ml)			
0.1	102 \pm 10	80 \pm 8	80 \pm 20
1	153 \pm 34	88 \pm 14	109 \pm 18
10	115 \pm 10	67 \pm 15	105 \pm 33
H9 shake			
Mock	228 \pm 35	85 \pm 14	148 \pm 29
HIV-1	273 \pm 52	79 \pm 16	160 \pm 41
HIV-2	164 \pm 36	85 \pm 15	127 \pm 46
H9 supernatant			
Mock	260 \pm 10	80 \pm 19	130 \pm 30
HIV-1	256 \pm 64	93 \pm 32	132 \pm 28

^a PBMC were cultured in ultrafiltered RPMI 1640 medium with 1% human serum at a concentration of 2.5×10^6 cells per ml. The cells were incubated with the indicated stimulus, and TNF- α , IL-1 β , and IL-6 were measured in the cell cultures 24 h later by using RIA. Each datum point is presented as the mean \pm standard error of the mean (SEM) for five separate experiments. Two experiments were performed with H9 supernatants.

Human Immunodeficiency Virus Does Not Induce Interleukin-1,
Interleukin-6, or Tumor Necrosis Factor in Mononuclear Cells

Jean-Michel Molina, David T. Scadden, Charlene Amirault, Annie Woon,
Edouard Vannier, Charles A. Dinarello, and Jerome E. Groopman

Journal of Virology: Planned to be published in June 1990

Table II

Table III

Properties of CD4 immunoadhesins and soluble rCD4									
	Calculated M_r	Subunit structure	gp120 binding (nM)*	Blocks infectivity		Plasma half-life in rabbits (hours)†	Fc binding (nM)*	Complement binding	Protein A binding
				T cells	M8				
rCD4	41,000	monomer	2.3 ± 0.4	Yes	Yes	0.25 ± 0.01	—	No	No
4y1	154,000	dimer	1.2 ± 0.1	Yes	Yes	6.7 ± 1.1	2.3 ± 0.7	No	Yes
2y1	112,000	dimer	1.4 ± 0.1	Yes	Yes	45.0 ± 5.6	2.6 ± 0.3	No	Yes
IgG1	145,000	tetramer (H ₂ L ₂)	—	—	—	113‡	3.2 ± 0.2	Yes	Yes

* Standard error of the mean was determined using the Inplot and Scatplot programs (see Fig. 3 legend). † Standard deviation indicated in hours.

‡ Determined in ref. 24. IgG1 has a half-life of 21 days in humans).

Designing CD4 Immunoadhesion for AIDS Therapy

Daniel J. Capon, Steven M. Chamow, Joyce Mordenti, Scot A. Marsters, Timothy Gregory, Hiroaki Mitsuya, Randal A. Byrn, Catherine Lucas, Florian M. Wurm, Jerome E. Groopman, Samuel Broder, and Douglas H. Smith.

Nature, 337:525-531, 1989

Table III